Pivotal Role of Increased Cell Proliferation in Human Carcinogenesis

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Cancer develops secondary to multiple genetic events. Each time a cell divides there is a rare chance that a genetic error related to the carcinogenic process will occur. Thus, environmental agents or disease processes that produce sustained increased cell proliferation can enhance the likelihood of cancer development by providing additional cell divisions, each with an opportunity for spontaneous genetic error. Studies of hereditary cancers and of various DNA-damaging agents, such as radiation and certain viruses and chemicals, have provided insight into identification of the essential genes, but many examples of carcinogenesis in humans do not involve direct DNA damage. Also, most preneoplastic lesions in human carcinogenesis show increased proliferation compared with normal tissues, whether from increased mitotic rate, blocked differentiation, prolonged cell survival, or other mechanisms. Selected examples of proliferation-related carcinogenesis are described, including certain infectious agents, defective immune surveillance, hormonal imbalances, chronic inflammatory-regenerative processes, and exposure to various chemicals. A common biologic mechanism for these diverse stimuli is increased cell proliferation as a prelude to cancer. This mechanism seems essential to the genesis of many cancers in humans.

Key words: Cell proliferation, Carcinogenesis, Viral cancer, Chemical carcinogens, Genetics, Immune surveillance.

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Cancer, the second leading cause of death in the United States, may be increasing, particularly in elderly individuals (1). Although progress has been made in the treatment of patients with cancer, prevention offers greater opportunities for reducing the death toll. Cigarette smoking, responsible for a majority of cancers of the respiratory tract and cancers of other organs, remains the leading known cause of cancer (2). Specific chemicals known to be carcinogens in humans, such as 2-naphthylamine, benzidine, 4-aminobiphenyl, vinyl chloride, and diethylstilbesterol, account for only a small percentage of cancers (3). Infectious organisms have also been implicated as being etiologic agents of specific cancers (4), including enteric bacteria, parasites, such as *Schistosoma* and *Clonorchis*, and viruses, such as Epstein-Barr virus (EBV), human T-lymphotropic viruses I and II, hepatitis B virus (HBV), and human papilloma virus (HPV).

Mounting evidence strongly supports the contention developed in 1914 that cancer results from genetic alterations (5). Utilizing molecular biologic techniques, numerous genetic alterations, including specific genes, have been identified in several cancers. However, many etiologic agents do not directly cause genetic damage. Similarly, some environmental agents associated with cancers do not directly damage DNA. Thus, although genetic damage is most likely an eventual common pathway to the development of cancer, other pivotal mechanisms contribute to carcinogenesis.

That multiple events are essential for the development of cancer has been demonstrated in experimental animal models, in *in vitro* systems, and in certain human cancers. Nearly 50 years ago, Berenblum and Shubik [6] conducted classical experiments in mouse cutaneous carcinogenesis that resulted in the formulation of the two-stage carcinogenesis concept. Alfred Knudson (7) hypothesized that two genetic events occur for retinoblastomas to emerge in children. His hypothesis has been confirmed through numerous genetic analyses and ultimately by the molecular cloning of a specific *Rb* gene.

Although cancer arises from defective control of cell proliferation, the etiologic and pathogenetic role of cell proliferation has received relatively little attention. Nevertheless, as early as 1953, Nordling (8) stated that, although genetic alterations were necessary, the likelihood that certain cancers would develop could be greatly augmented by sustaining cell prolifer-

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ation of the target tissue. A decade ago, a specifically define role for cell proliferation was integrated into a carcinogenesis model developed by Moolgavkar and coworkers (9, 10), which was derived from epidemiologic data, and into a biologically similar model formulated by Greenfield *et al.* (11) and by Cohen and Ellwein (12), using data from animal experiments. Although derived from two different perspectives, the biologic framework of both models is strikingly similar. They offer a basis for interpreting a wide variety of carcinogenesis data in animal models and humans. Both models quantify genetic and proliferative events and thus offer insight into assessments dealing with the risk of developing cancer. The framework of these models is presented below and then selectively illustrated in human carcinogenesis. We have attempted to identify the common biologic thread of increased cell proliferation as a common prelude to carcinogenesis. This perspective is not intended to be definitive and, thus, the important work of many investigators is not cited nor is the burgeoning information being published regarding multiple molecular events being discovered for specific histologic types of cancer.

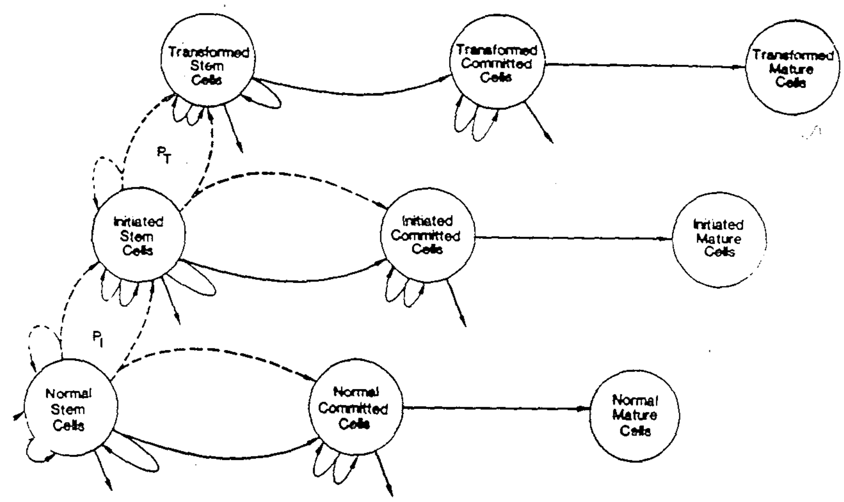

Figure 1. Diagrammatic representation of the biologic model of carcinogenesis originally described by Greenfield *et al.* (11). The *bottom three circles* represent the normal differentiation of a tissue. Ascending along the *left side* are the two stages of carcinogenesis, initiation and transformation. *Downward-pointing arrows* represent cell death, whereas the *other arrows* represent the various combinations of possible results of cells following cell division.

CELL PROLIFERATION AND CARCINOGENESIS

The model of carcinogenesis discussed and illustrated herein is shown in Fig. 1. For any theoretic model, assumptions are made in defining qualitative and quantitative aspects. The assumptions of this model are the following: (*a*) cancer arises from normal cells through two irreversible genetic events; (*b*) these genetic events occur only during active cell proliferation or are irreversibly fixed only during cell division; (*c*) the carcinogenic events occur only in a susceptible subpopulation of cells within the target tissue (frequently referred to as stem cells); and (*d*) the two genetic events occur in a random fashion with non-zero spontaneous probabilities. Note that the word "transformation" is used to mean the development of *malignant* cells.

In the context of this model, an agent can alter the likelihood of developing a cancer in only two ways: it can increase the probability of irreversible genetic damage occurring during cell division; or it can increase cell proliferation, usually accompanied by an increase in cell number, and consequently increase the number of opportunities for spontaneous genetic damage. It also can do both. Although other models postulating more than two critical genetic events in the carcinogenic process have been proposed, our analyses reveal that two critical events seem to be sufficient for cancer to occur. We recognize that the size of the susceptible population of cells and their susceptibility can be altered by a variety of genetic and nongenetic events and stimuli. Also, further genetic alterations may occur in subclones of a malignancy, producing considerable heterogeneity with respect to several aspects of its biologic behavior. Clearly, other events occur during progression of cancers that endow the malignancies with increased survival advantage.

Under normal circumstances, the probability for either of the two critical genetic events to occur is exceedingly low (probably in the range of 10^{-10} to 10^{-6} per cell division); otherwise everyone would develop cancer at an early age. On the other hand, these probabilities are not zero, or no one would develop cancer. Thus, this model predicts that, if people lived sufficiently long, all would develop cancer. However, because these probabilities are so low, the odds are in favor of an individual not developing cancer, even with a life span of 100 yr. Approximately 25% of persons develop malignancy in the United States during their lifetime.

INHERITED CANCERS

Studies of hereditary cancers of children have provided experiments of nature illuminating how carcinogenesis can occur. As originally advanced by Knudson (7), genetic events occur in the two alleles of the *Rb* gene that give rise to retinoblastoma (Fig. 2). Normally, the likelihood of developing retinoblastoma in an individual without an inherited retinoblastoma gene defect is rare, given that two rare events are required for the tumor. In contrast, individuals who inherit the defect in the *Rb* gene have nearly a 100% occurrence of the tumor. Although this phenotypic expression initially suggested a dominant trait, Knudson postulated autosomal recessive *Rb* gene inheritance. With retinoblastic proliferation during development, a genetic error eventually occurs in the second *Rb* allele. Although rare during any one mitotic event, the

probability that a mutation will occur is sufficiently high that nearly all genetically susceptible individuals develop retinoblastoma. Incidences frequently are bilateral, and/or persons develop more than one tumor per eye at an early age.

Retinoblasts only proliferate during development of the eye, and cell division is necessary for either of the two genetic events in the genesis of retinoblastoma to occur (unless one allele is defective because of a germ line mutation). Thus, the chance of developing a retinoblastoma is eliminated once these cells stop proliferating. Similar arguments can be advanced for neuroblastoma, since neuroblasts also cease proliferating during childhood.

Knudson's hypothesis prompted the search for other tumor suppressor genes (also referred to as antioncogenes) (13, 14). Increased susceptibility to the development of tumors in other tissues, such as osteogenic sarcomas, has been observed in patients with retinoblastoma, although it remains unclear as to why tumors do not increase in all tissues. A second suppressor gene (with protein product p53) might be involved with the genesis of these sarcomas. Other possible candidates for tumor suppressor genes include Wilms' tumor, renal cell carcinoma, and at least two forms of inherited colonic carcinoma.

Polyposis coli (Pc) is an autosomal dominant, inherited susceptibility to adenomatous polyps and adenocarcinoma of the colon (15). Individuals with the Pc genetic defect (chromosome 5q) develop numerous colonic polyps that often evolve into carcinomas within a few decades. Similar genetic events occur in some nonpolyposis coli patients, who more commonly develop colon cancer at a later age. In addition, at least six other autosomal dominant hereditary traits predispose to colon cancer (16).

Adenomatous polyps, which are preneoplastic lesions, exhibit increased proliferative capacity, presumably due to enhanced proliferation of the colonic crypts. Most (if not all) preneoplastic lesions involved in human carcinogenesis show increased proliferation compared with normal tissue, whether it comes from increased mitotic rate, blockage in differentiation, or other mechanisms.

Fearon and Vogelstein (17) have recently postulated a multistep process for colonic carcinoma. However, their multiple stage model could also be consistent with only two critical events being required for carcinogenesis (Fig. 3). The additional genetic alterations that they observe in other genes may enhance the proliferative capacity or alter the differentiation of cells in the preneoplastic, adenomatous polyp. Although these

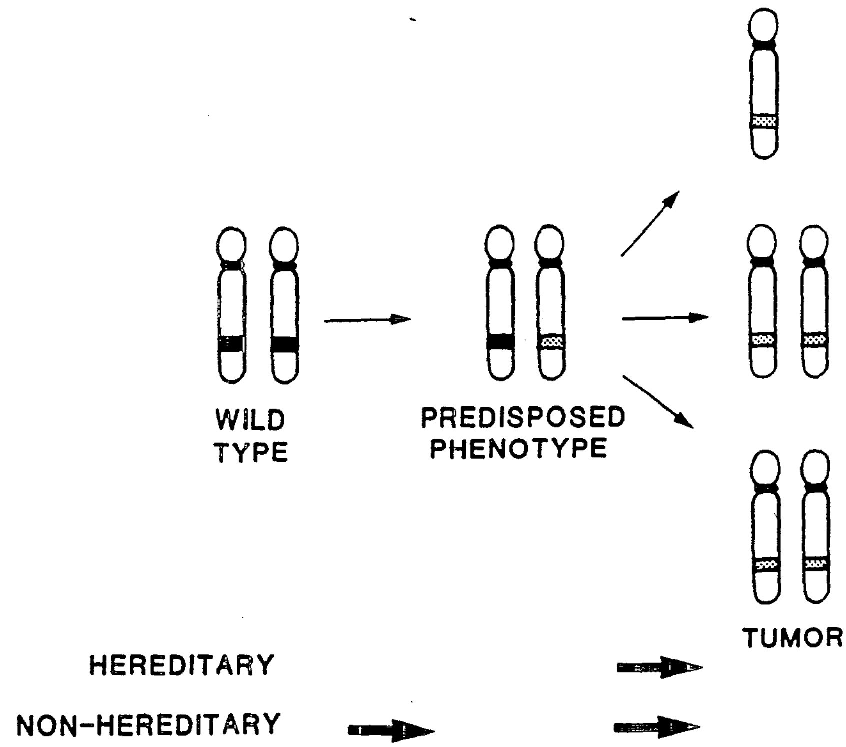

Figure 2. Genetics of retinoblastoma. Tumors occur when defects occur in both alleles, whether caused by absence of the entire chromosome, deletion of a portion or all of the gene segment, or mutation of the gene. Individuals with hereditary retinoblastoma are born with one defective allele in all of their cells, requiring only a defect to develop in the second allele for malignancy to occur. Nonhereditary individuals must generate defects in both alleles beginning with cells having two normal alleles at conception.

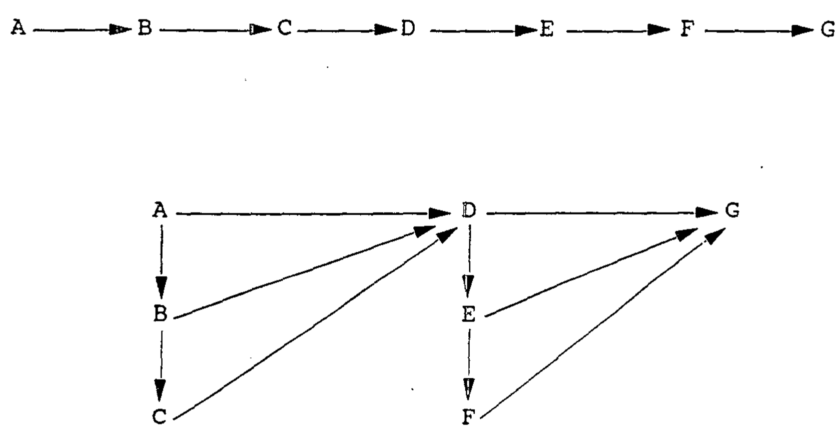

Figure 3. Alternative explanations of multiple genetic events occurring during carcinogenesis. If each of the identified genetic events occurs sequentially, the *upper diagram* pertains. However, more likely is a mechanism similar to that presented in the *lower diagram* where there are multiple genetic events that will affect the proliferative rates, genetic stability, and/or the cell population sizes of *A*, *B*, or *C*, but are not essential for the carcinogenic process itself. However, these additional genetic effects will greatly accelerate the carcinogenic process overall.

secondary genetic alterations may give a proliferative advantage to preneoplastic cells, and may thus significantly decrease the time to the development of an actual malignancy, they nevertheless are not required, rate-limiting events in the development of the tumor.

HORMONES AND CANCER

Hormones govern numerous cellular functions, including proliferation, growth, and maintenance of bodily functions. Clinical and epidemiologic studies demonstrate that sustained hormonal stimulation (18) and consequent enhanced cell proliferation result in estrogen-dependent endometrial (19) and breast carcinomas (20), thyroid-stimulating hormone (TSH)-dependent thyroid tumors (21), and androgen and estrogen interactions in the development of prostatic cancer (22).

Endometrial carcinoma frequently results from chronic estrogen stimulation of cellular proliferation. For example, an increased incidence of endometrial adenocarcinomas results from exogenous estrogen therapy, such as seen with hormone replacement for menopausal women (19) and, possibly, from the use of older-type, estrogen-containing contraceptives (23). In addition, obesity is a risk factor for endometrial carcinoma, possibly due to hyperestrogenism from increased production or storage of estrogen by adipose cells (24). Moreover, chronic estrogen stimulation is associated with endometrial hyperplasia and carcinoma in women with the polycystic ovary syndrome (25). Characteristically, estrogen stimulates the endometrium to proliferate (Fig. 4). Normally, this proliferative stimulus is tempered in midcycle by the increased production of progesterone, ultimately resulting in the shedding of cells during menstruation. In the circumstances described above, estrogen stimulation is sustained rather than cyclic.

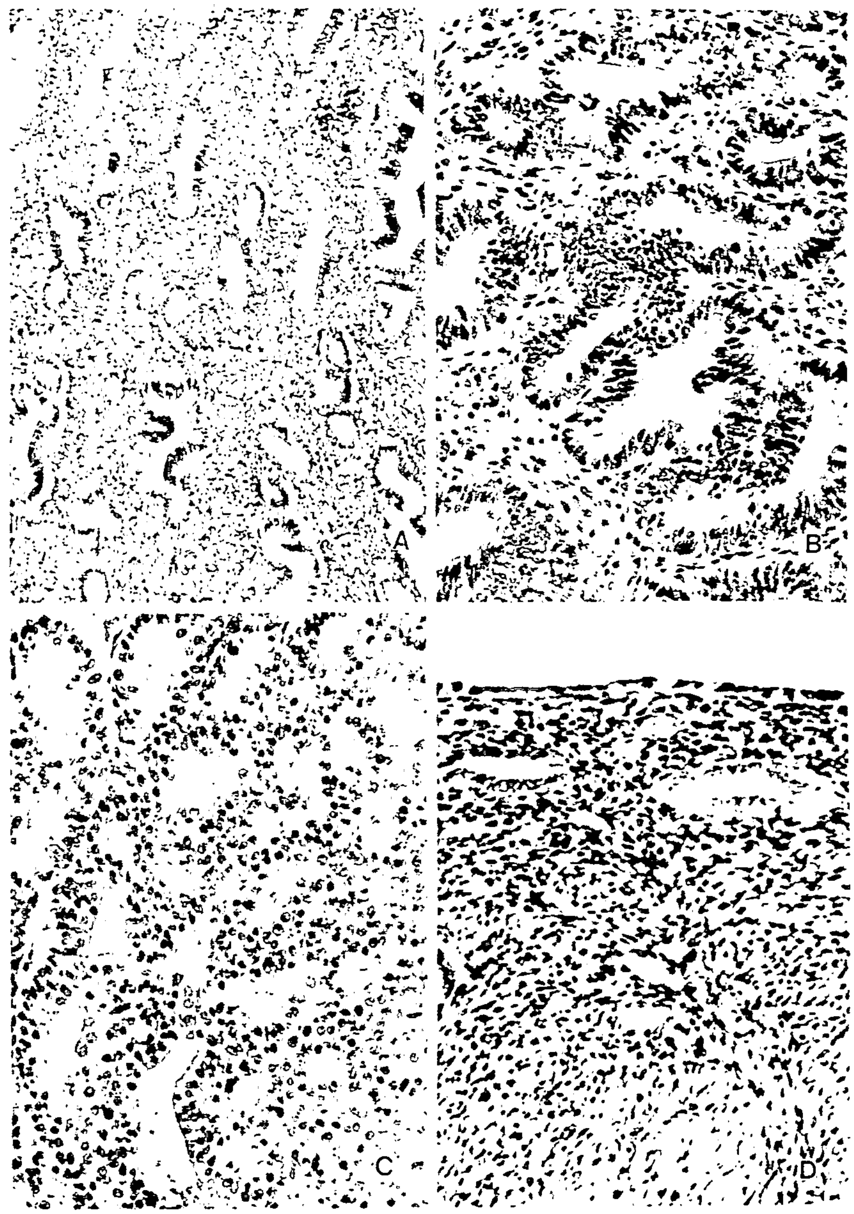

Figure 4. Estrogens have a proliferative effect on the endometrium. During the normal reproductive stage of a woman's life, this produces a proliferative endometrium (*A*), which is converted to secretory endometrium by progestational effects. However, if there is hyperestrogenism secondary to exogenous or endogenous sources, a hyperplastic endometrium results (*B*), since the proliferative effects of estrogen are not impeded by the normal or subnormal levels of progestins. If the adenomatous hyperplasia continues, a malignant tumor, adenocarcinoma, arises (*C*). This is particularly striking in postmenopausal women who have hyperestrogenism secondary to exogenous sources, but also can occur secondary to endogenous production. In contrast, postmenopausal women usually have lost the proliferative-stimulatory effects of estrogen, and their endometrium becomes atrophic (*D*).

Estrogen-related substances, such as diethylstilbesterol (DES), stimulate estrogen-responsive cells. In experimental animals, DES induces a variety of estrogen-related tumors (26). In humans, the development of vaginal adenocarcinomas in the offspring of mothers who were exposed to DES during pregnancy is a notorious example (27). DES given to experimental animals, particularly in the hamster kidney model, undergoes metabolic activation and DNA adduct formation (28). However, the principal role of DES in tumorigenesis appears not to involve DNA adduct formation but increased cell proliferation of estrogen-responsive cells (29). The situation in the human may reflect both of these components. The initial event *in utero* is likely due to interaction of DES with the DNA of specific vaginal cells. These initiated cells undergo rapid cell proliferation following

menarache, often leading to the carcinogenic second event.

The role of estrogen, and possibly other hormones, in causing breast cancer is similar to that in the endometrium (20). However, other critical factors may affect the responsiveness of cells to estrogen stimulation. For example, the age at which a woman has her first child significantly influences her susceptibility to breast cancer: the younger the woman is at the time of her initial pregnancy, the less likely she is to develop breast cancer. During pregnancy, terminal differentiation of the breast ductules occurs, removing a large number of cells from the cancer-susceptible population. Thus, even if these cells are subsequently stimulated to increased proliferation, they are not susceptible to developing cancer. Human chorionic gonadotropin (HCG) also appears to be involved with the induction of this differentiation process. An initial pregnancy at a later age prolongs the susceptible period of these cells. Not only is the rate of cell proliferation important, but the size of the susceptible cell population and the period of time over which it persists influence the chances for experiencing the critical events necessary for developing cancer.

Chronic increased cell proliferation induced by estrogen also increases the appearance of benign and malignant hepatocellular tumors in experimental animals and in humans (30). Hepatocytes with estrogen receptors respond to increased estrogen levels by dividing more frequently.

Hormonal effects on cell proliferation also greatly affect the likelihood of developing prostatic adenocarcinoma (22). Animal models have been developed wherein the prostate is provoked into a burst of cell proliferation. This proliferation can then be hormonally sustained, leading to the development of adenocarcinomas. In these instances, it remains unclear what the interactions between androgens and estrogens are, but both and possibly other hormones appear to be involved.

Thyroid carcinogenesis involves the interaction between the thyroid and the pituitary in a feedback loop (18, 21). As the thyroid produces more thyroid hormone (T_3 or T_4), it inhibits the pituitary, reducing TSH production. If thyroid hormone levels decrease, TSH levels produced by the pituitary increase, resulting in increased thyroid proliferation. In animal models, this process is frequently seen following the administration of chemicals (21) that decrease levels of thyroid hormone by a variety of mechanisms. This ablates negative feedback on the pituitary and, consequently, overproduction of TSH results and thyroid follicular cell proliferation arises. Ultimately, thyroid tumors result. Again, there is no evidence that TSH damages DNA by itself; these tumors arise as a consequence of chronic increased cell proliferation of the target tissue. This mechanism is nongenotoxic, but a thyrotoxic chemical can ultimately evoke tumors in the target organ.

INFECTIOUS ORGANISMS AND CANCER

Several microbial agents increase cell proliferation and increase the risk of developing cancer. The intriguing possibility that infectious organisms might cause cancer has been investigated for more than a century. The first transmissible carcinogenic viral agents were identified in experiments by Rous and by Ellerman and Bang during the early part of this century (31). Cell-free extracts were found to transmit cancer from diseased to disease-free animals.

Some fungi have been implicated in cancer development by producing specific carcinogenic toxins, for example, aflatoxin (32). Bacteria have also been associated with the production of carcinogenic chemicals. For example, enteric bacteria occasionally are involved in the metabolic activation of certain carcinogens, such as cycasin (33). Other organisms more directly cause specific cancers.

Immunoproliferative small intestinal disease (IPSID) is found in males in Third World countries. The initial benign appearing hyperplastic lymphoid lesion is thought to arise from chronic antigenic stimulation by bacterial lipopolysaccharides or enterotoxins of *Vibrio cholerae*. Supporting this view is the regression of the lesions following a 6-mo trial of tetracycline (34). Without treatment, these lesions can convert to monoclonal malignant lymphoma that secretes α heavy chains of immunoglobulin.

Certain parasitic diseases increase susceptibility to cancer most notably schistosomiasis (35) and clonorchiasis (36). Chronic *Schistosoma hematobium* infection is associated with a markedly increased risk of developing bladder cancer. This agent causes chronic inflammation, fibrosis, squamous metaplasia, and sustained, increased, squamous cell proliferation compared with the normal, mitotically quiescent transitional epithelium (Fig. 5). The majority of the tumors that develop within these infected bladders are squamous cell carcinomas, rather than the usual transitional cell carcinomas. Although specific carcinogens, such as nitrosamines, may be produced in schistosomiasis, sustained increased cell proliferation is pivotal to generating these tumors.

Schistosomal infections of the lower gastrointestinal tract (*S. mansoni* and *S. japonicum*), common in the Far East and elsewhere, are associated with development of colonic carcinomas (37). This association is considerably less frequent than with schistosomiasis and bladder cancer. Again, sustained increased proliferation of the colonic epithelium may be a mechanism responsible for these cancers.

Chronic biliary tract infections with the flukes, *Clonorchis sinensis* (36) or *Opisthorchis viverrini* (38), evoke destruction, epithelial regeneration, and an increased prevalence of cholangiocarcinoma (Fig. 6). A specific carcinogen is not implicated in this process, whereas increased cell proliferation is sustained in bile ducts and ductules.

Although on a worldwide basis these infections and tumors are common, they seldom affect persons in economically developed countries. In contrast, specific RNA and DNA viruses infecting populations globally can be carcinogenic (31, 39).

Figure 5. Squamous cell metaplasia (*A*) of the urinary bladder in a patient with chronic schistosomiasis. There is also ulceration of the epithelium. Note the numerous schistosome organisms in the wall of the bladder. The organisms can also be seen in the poorly differentiated squamous cell carcinoma that arose in another patient with schistosomiasis (*B*).

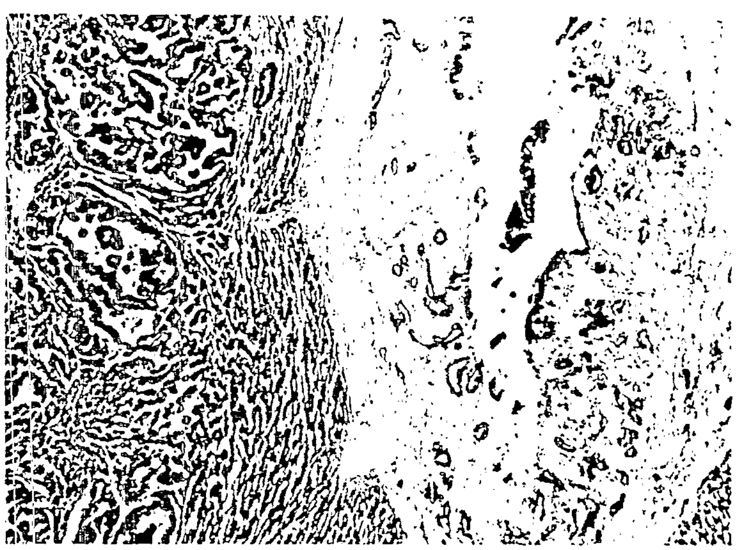

Figure 6. Bile ductular proliferation (*right*) secondary to *Clonorchis* infection, which has given rise to a cholangiocarcinoma (*left*).

Numerous oncogenic RNA retroviruses affect animals. Retroviruses convert viral RNA to DNA by utilizing the reverse transcriptase enzyme; the DNA is then incorporated into the genome of the host. It has been hypothesized that specific viral oncogenes have been incorporated into the human genome as protooncogenes or cellular oncogenes (39, 40). These viral and human cellular homologs act in a genetically dominant fashion. The Rous sarcoma virus carries the viral *src* gene. Several other viral oncogenes of retroviruses that infect humans have been identified and can result in leukemia or lymphoma.

Other retroviruses can produce cancers without carrying a specific oncogene as part of their RNA (39, 40) by increasing the proliferation of the target tissue. Transmission of virus occurs from cell to cell, eventually resulting in the interposition of virally generated DNA next to a cellular oncogene. Thus, sustained cell proliferation and, ultimately, tumors can arise. A single oncogene, such as *bcl-2* involved in follicular lymphoma, appears incapable of producing cancer without a second event. This oncogene, which is activated by the reciprocal translocation t(14;18) involving the breakpoint at *bcl-2* on chromosome 18 and the heavy chain locus at 14q32, enhances follicular center cell proliferation. A second event is needed for the malignant counterpart to emerge.

The human oncogenic retrovirus, human T-cell leukemia virus (HTLV) I, chronically infects nearly 1 million people in Japan. Given that only 400 patients develop adult T-cell leukemias yearly in Japan, it is evident that a multistep process prevails. It has been postulated that immune deficiency and genetic events are involved in this leukemogenicity (41).

Infection with human immunodeficiency virus (HIV) results in an increased susceptibility to malignant lymphomas, squamous cell carcinomas, and Kaposi's sarcoma, but does not seem to be directly oncogenic (42) (see below under "Immune Surveillance of Cancer").

Several DNA viruses, including hepatitis B virus (HBV), human papilloma virus (HPV), and Epstein-Barr virus (EBV), are associated with certain types of cancers in humans. In each instance, the development of the malignancies results from a sustained proliferation of the target cell. Herpes viruses I and II have also been associated with an increased risk of cervical cancer, although this association has not been confirmed by recent research (43).

HBV infection is mostly asymptomatic and transient. However, in susceptible individuals, the acute infection leads to sequelae of chronic active hepatitis (Fig. 7), which can progress to cirrhosis (44). Likely, the virus persists predominantly in males owing to an inadequate immune response to the virus. The male:female ratio of primary hepatomas is 4:1. Females have superior immunocompetence to HBV than do males, as has been shown in studies done in Taiwan (45). Increased androgens may also enhance hepatocarcinogenesis.

The characteristic features of chronic active hepatitis and cirrhosis are hepatocellular necrosis simultaneously with regenerative repair. The normal liver is a mitotically quiescent tissue as is the urinary bladder epithelium. With chronic active hepatitis or cirrhosis, hepatocyte proliferation is markedly increased and is sustained for the life of the patient. In a significant number of such patients, hepatoma arises. Worldwide, hepatomas are caused primarily by chronic HBV (46).

HBV-related hepatocarcinogenesis is probably not related directly to a specific oncogenic DNA alteration induced by the virus itself. Transgenic mice that overproduce the large envelope polypeptide of HBV accumulate hepatitis B surface antigen and develop chronic active hepatitis, regenerative nodules, and ultimately hepatomas (47). This protein has none of the characteristics of oncogenes or tumor suppressor genes, but rather appears to be involved with the development of hepatocellular necrosis, chronic active hepatitis, and sustained, increased hepatocyte proliferation.

Any situation resulting in a chronic inflammatory or cirrhotic process is associated with an increased proliferative rate, regenerative nodules, and an increased risk of hepatomas. Examples include chronic alcoholism and a variety of hereditary disorders, such as hemochromatosis. Not one of these conditions causes specific genetic damage, but they have in common increased sustained cell proliferation.

HPV infects squamous epithelia and is most commonly associated with cervical squamous cell carcinoma. Also, squamous cell carcinomas of the penis, skin, anus, and oral cavity frequently contain the virus (48). HPV blocks differentiation of the infected epithelium, giving features of dysplasia (Fig. 8). Increased cell proliferation and expansion of the basal cell compartment result. Since HPV infections are usually persistent, events leading to the continued presence of dysplasia can evolve, occasionally leading to carcinoma *in situ* and squamous cell carcinoma (49). Again, HPV causes a greatly increased risk of carcinoma owing to enhanced cell proliferation. Smoking cigarettes and defective immune responsiveness to the virus also appear to play a role (48, 50).

EBV is a well-known B-cell mitogen. The virus infects B-cells through the C_3d (CR_2) receptor and immortalizes them *in vitro*. During acute infectious mononucleosis (Fig. 9), approximately one per 10^4 B-cells is infected, whereas during latency approximately one per 10^6 B-cells is infected. Multiple immune responses, especially by T-cells, bring the B-cell proliferation under control (51). However, if the polyclonal B-cell proliferation is not brought under control, Burkitt's or other non-Hodgkin's lymphomas can arise. Immune-deficient individuals chronically immunosuppressed by holoendemic malaria, children with inherited immunodeficiency, HIV-infected persons, or transplant recipients frequently develop EBV-carrying tumors.

Klein and Klein (52) postulated a multistep scenario in the genesis of African Burkitt's lymphoma. Holoendemic malaria suppresses cytotoxic T-cells against EBV-infected B-cells while simultaneously causing polyclonal B-cell prolifer-

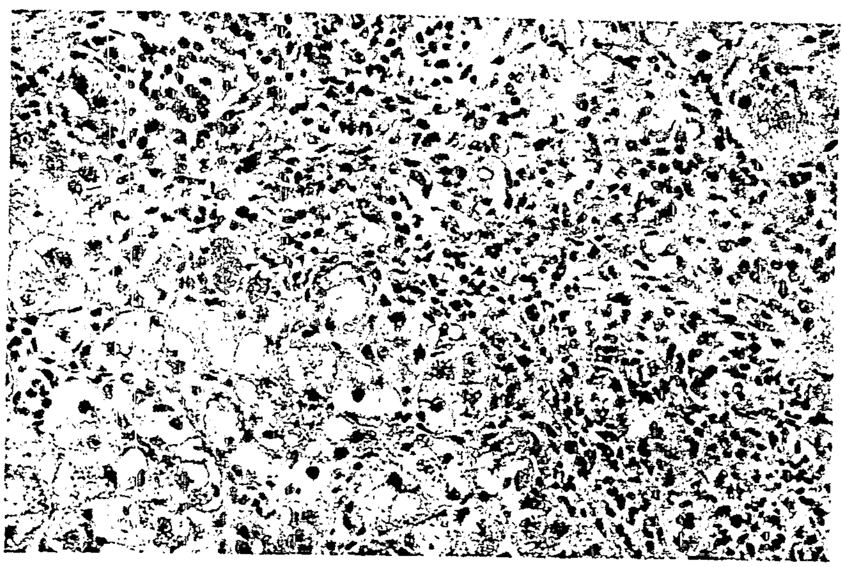

Figure 7. Chronic active hepatitis secondary to HBV infection. This is a chronic necroinflammatory process with sustained regeneration, occasionally leading to the development of hepatoma.

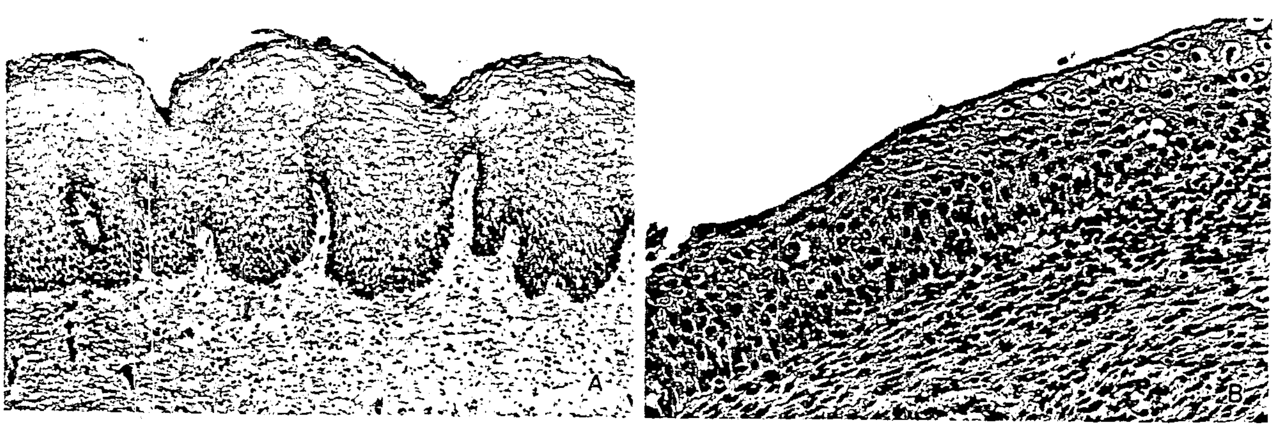

Figure 8. The normal squamous epithelium of the cervix shows a "stem cell" basal layer with differentiation progressing to the surface (*A*). With HPV infection, there is blockage of this differentiation process leading to an expansion of the proliferative pool of cells extending higher in the epithelium, above the basal layer (*B*). In this figure, there is clear evidence of HPV infection as indicated by the koilocytes and chronic inflammation, with increasing degrees of dysplasia progressing from *right* to *left*.

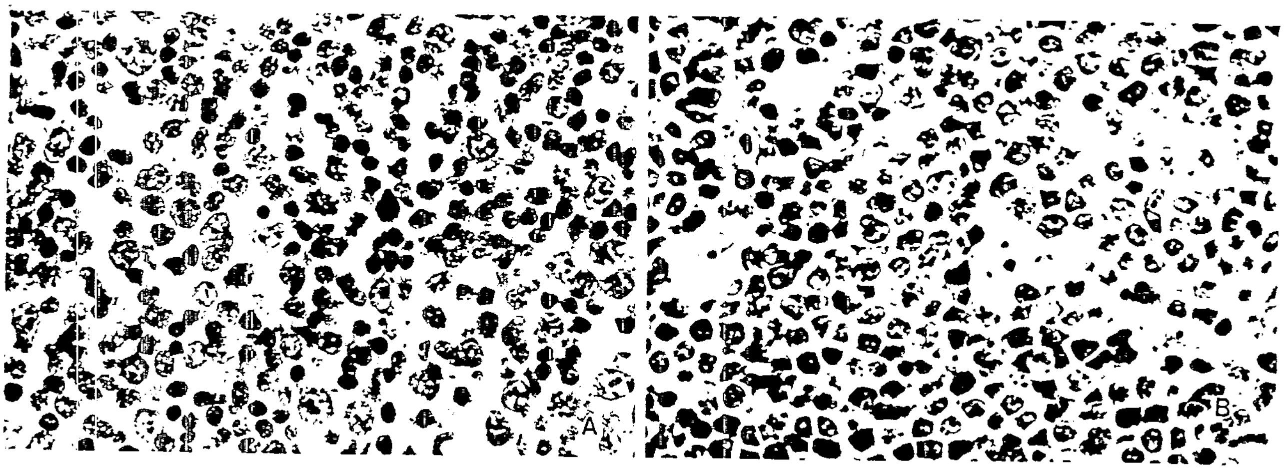

Figure 9. Infectious mononucleosis (*A*) is a markedly proliferative acute infection secondary to EBV, which is normally brought under control by various immune factors. In immunosuppressed patients, this control of B-cell proliferation does not occur. The sustained proliferation can eventuate in the development of B-cell lymphomas, such as Burkitt's lymphoma (*B*).

ation. This increased cell proliferation increases the random chance that a specific chromosomal translocation might occur involving the c-*myc* protooncogene at 8q24 with corresponding breakpoints involving immunoglobulin loci (14q32, 2p12, or 22q11). Juxtaposition of an *Ig* gene with c-*myc* promotes expression of the c-*myc* gene product. Concurrently, the major histocompatibility complex (MHC) and EBV viral targets for T-cell surveillance are down regulated. Identical chromosomal translocations are seen in mouse and rat immunocytomas and plasmacytomas. Moreover, the *Ig-myc* transgene results in transgenic mice that develop pre-B-cell malignant lymphomas (53).

In cell culture systems, EBV readily produces a mitogenic response in B-cells but does not result in the production of malignant transformation (54). This suggests that EBV does not have a specific malignant transforming gene and that the specific chromosomal translocation leading to Burkitt's lymphoma is an exceedingly rare event. The probability that any particular cell division will produce a malignant transformation is not increased, but, in the patient wherein uncontrolled polyclonal B-cell proliferation persists, the number of cell divisions is enormously increased. The odds of a chromosomal translocation occurring within the susceptible pre-B-cell population are thus increased.

IMMUNE SURVEILLANCE OF CANCER

In 1957, Burnet proposed the notion that the immune system routinely recognizes and eliminates newly generated cancer cells. This hypothesis was extended by Thomas in 1959 (see Ref. 55). Cancer was proposed to arise chiefly because of a breakdown in the immune surveillance against cancer cells. This theory was based on several experimental observations. In mice, tumor-specific antigens were identified, suggesting that tumor cells had specific antigens that could be detected by the immune system and eliminated. Furthermore, several viral and chemical carcinogens were demonstrated to have immunosuppressive properties in animal models. Further, supporting this theory, clinical observations indicated that patients with congenital immunodeficiencies, such as Wiskott-Aldrich syndrome, or acquired immunodeficiency secondary to immunosuppressive therapy for renal transplantation had a markedly increased occurrence of malignancies.

Although superficially plausible, additional observations and experimentation have revealed that the immune surveillance theory of carcinogenesis is not correct. The tumor-specific antigens that were discovered in mice were determined to be related primarily to tumorigenic viruses or H-2 antigens. Tumor-specific antigens in human cancers have only been discovered in multiple myeloma (56). A wide variety of tumor-associated antigens have been identified, which are embryonic or differentiation antigens of normal cells. Although qualitative differences have not been identified, quantitative differences between normal cells and cancer cells have been demonstrated.

The immunosuppressive effects of a variety of carcinogens, particularly the polycyclic aromatic hydrocarbons, and some of the carcinogenic viruses are immunosuppressive only at doses far in excess of those known to cause cancer, or they are immunosuppressive using routes of administration unrelated to the carcinogenicity studies (57). For other chemicals or viruses shown to be carcinogenic in various animals, immunosuppressive properties could not be demonstrated. Also, many experimental models were shown to be unaffected when immunosuppressants, such as azathioprine or cyclophosphamide, were administered concurrently or sequentially with the carcinogenic agent (58).

Although a marked increased prevalence of malignancies occurs in immunocompromised patients (59), all types of malignancies are not increased; only B-cell lymphomas, Kaposi's sarcoma, and cutaneous, oral, anal, and uterine cervical squamous cell carcinomas (Fig. 10) are increased (59, 60). As

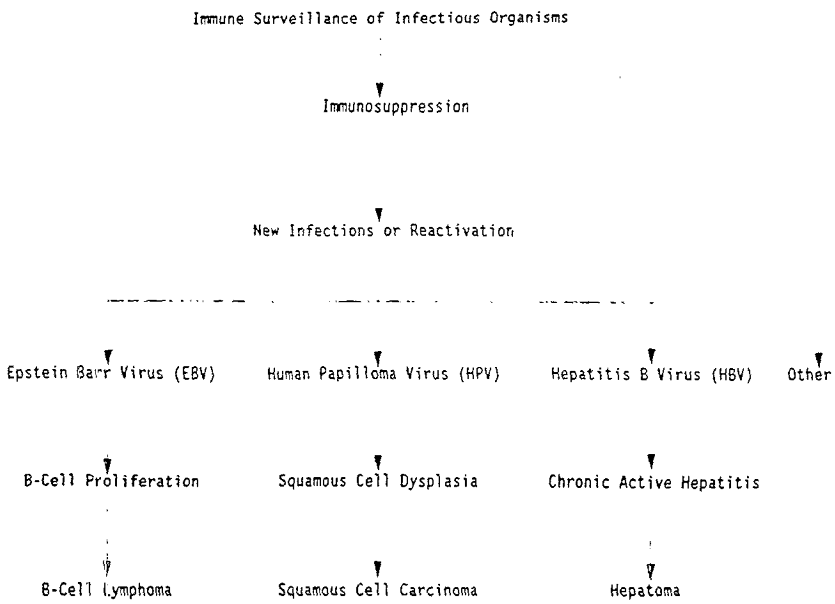

Figure 10. Diagrammatic representation of the role of immune surveillance of various infectious organisms in the etiology of specific cancers. The immune system normally controls the acute infection. If the patient is immunosuppressed (hereditary, transplantation, AIDS), chronic proliferative effects ensue from the uncontrolled infections, occasionally resulting in malignancy.

described above, these malignancies have been associated with viruses. EBV is present in the B-cell malignancies and HPV in the squamous cell carcinomas. Kaposi's sarcoma occurs in AIDS patients, possibly arising from the *Tat* gene of HIV or growth factors liberated by stimulated T-cells, or other, yet to be identified, factors (61). The common biologic theme among these tumors is a chronic increased proliferation of the target cells resulting from persistent, new, or reactivated viral infections. Immunity controls the extent of production and persistence of these viruses.

Thus, immune surveillance is germane to carcinogenesis with these specific, virus-induced tumors. Surveillance, however, is not against malignant cells but against the infectious organisms. As indicated above, excellent examples of this are the EBV-induced lymphoid malignancies in immunosuppressed patients that result from a failure of T-cells to recognize Epstein-Barr viral antigens on the surface of infected B-cells and to eliminate them. The sustained cell proliferation leads to a substantially increased risk of cancer.

Although there is little evidence to support the immune surveillance theory of carcinogenesis as originally described in the 1950s and 1960s, immune surveillance is plausible for microbe-induced cancers that act primarily through producing mitogenesis. The immune system under Darwinian evolutionary pressures evolved to protect the host against life-threatening infections and not cancer. Malignancies occur largely during the postreproductive period and, thus, natural selection would not occur. However, immunologic regulation of the invasiveness and metastatic potential of cancers, such as melanomas, bladder carcinomas, leukemia, lymphoma, and renal cell carcinomas, may be important (62).

CHRONIC INFLAMMATORY PROCESSES

As summarized above, many chronic inflammatory processes increase the risk that cancer might develop. In addition, in the gastrointestinal tract, notable examples are the association of chronic atrophic gastritis with gastric carcinoma (63) and chronic ulcerative colitis with colonic carcinoma (64). A chronic necroinflammatory process results in sustained regenerative proliferation of cells that gain a proliferative, and possibly a survival, advantage greater than the surrounding normal tissue. Gastric intestinal metaplasia or colonic epithelial dysplasia ensues that can develop into proliferative foci, adenomatous polyps, and adenocarcinomas.

The presence of agents that enhance the proliferative process, such as high salt intake or *Helicobacter* infections associated with the stomach (63, 65), or bile acids, high fat, and low fiber diets with the colon (66), predisposes to development of cancer. Conversely, dietary calcium, high fiber, and low fat are associated with decreasing colonic cancer risk by decreasing proliferation (66, 67).

Gallstones and gallbladder and biliary tract cancer (68), tropical phagedenic ulcer and squamous cell carcinoma of the skin (69), and chronic esophagitis secondary to gastric reflux leading to Barrett's esophagus and adenocarcinoma (70) are other situations characterized by sustained cellular proliferation and frequent carcinogenesis (Table 1).

In a similar vein, high rates of growth of normal tissues are also associated with an increased risk of cancer. For example, osteogenic sarcoma incidence peaks during adolescence when growth is marked (71). Osteogenic sarcomas in older individuals are frequently associated with Paget's disease, a disease associated with an increased proliferative process of the osteoblasts (72).

CELL PROLIFERATION AND CHEMICAL CARCINOGENS

Numerous chemicals and chemical mixtures increase the risk of developing cancer, including cigarette smoking, snuff use, betel quid chewing, aromatic amines, polycyclic aromatic hydrocarbons, nitrosamines, and others as detailed in a recent IARC monograph (26). Most of these chemicals are both mutagenic in short-term screening assays and carcinogenic in a variety of species. They are also cytotoxic to the target tissue, resulting in regeneration and increased cell proliferation (Fig. 11). At toxic doses, a sharp increase in the rate of tumor formation is observed.

TABLE 1. SOME OF THE CHRONIC CONDITIONS ASSOCIATED WITH INCREASED CELL PROLIFERATION AND INCREASED RISK OF CANCER DEVELOPMENT

Organ	Chronic Condition
Skin	Phagedenic ulcer
Esophagus	Reflux esophagitis with Barrett's esophagus
Stomach	Chronic atrophic gastritis
Colon	Chronic ulcerative colitis
Liver	Cirrhosis
Gallbladder	Cholelithiasis
Bone	Paget's disease

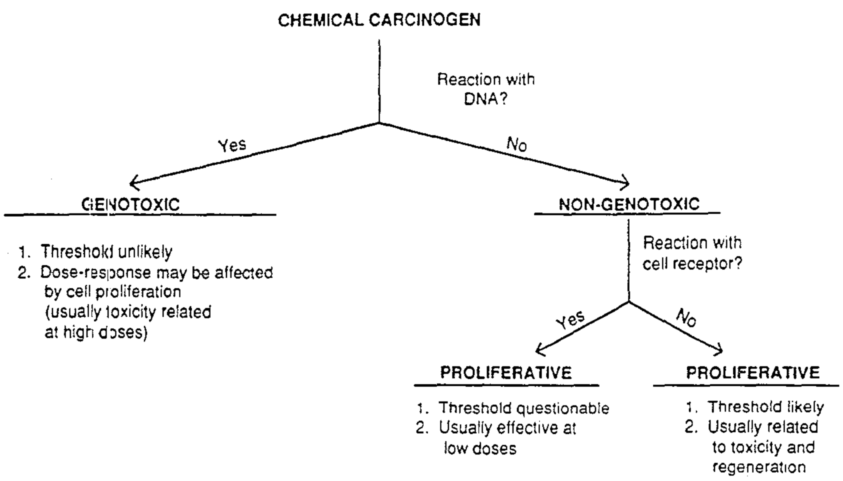

Figure 11. Diagrammatic representation of proposed classification of chemical carcinogens based on their ability to react directly with DNA or cell receptors. Cell proliferation affects the dose-response of all classes of chemical carcinogens [From Cohen and Ellwein (12)].

Similarly, UV radiation is associated with skin carcinomas (73); high energy radiation with cancers of the bone marrow (leukemia), thyroid, breast, and other tissues (73); and thorotrast with liver and kidney cancers (74). These forms of radiation are obviously genotoxic, but the development of tumors secondary to radiation is frequently associated with chronic, destructive-regenerative processes.

Although the carcinogenic synergism associated with the proliferative effects of chemicals has been best documented in experimental animals, this likely holds for humans also. For example, cigarette smoke is toxic to the respiratory epithelium, leading to chronic bronchitis and squamous metaplasia, associated with increased cell proliferation (75). In addition, cigarette smoking produces hyperplasia and carcinoma of the urinary bladder (76). Snuff and other orally used tobacco products contain many carcinogens, but they are also associated with chronic inflammatory, regenerative processes in the oral cavity and pharynx (77). Likely, the combination of their genotoxicity and increased cell proliferation results in cancer in these tissues in humans.

When tested in experimental bioassays for carcinogenic activity, a large proportion of chemicals that are negative in various short-term mutagenicity screens show carcinogenic activity in mice and/or rats (78). This raises concern regarding the interpretation of these data and the extrapolation of potential risk to humans for compounds in the environment or food supply. A major complication in assessing risk from these chemicals is that tumors usually occur only at high, often toxic, doses that are frequently associated with increased cell proliferation of the target tissue (12).

For nongenotoxic chemicals that demonstrate proliferative effects only at very high doses, a no-effect threshold might exist (12). For example, when melamine is administered to rats or mice at high doses, urinary calculi form, and ultimately, bladder tumors develop (12, 79, 80). When lower doses of melamine are administered and calculi do not form, cell proliferation is not increased and no tumors form. The data regarding melamine and related compounds that induce calculi in experimental animals only at high doses imply that there is no carcinogenic risk for humans exposed at low doses, where calculi do not form. The Environmental Protection Agency (EPA) has recently followed this pragmatic logic and an understanding of biologic mechanisms in interpretating data for melamine (81).

Another example with a similar mechanistic action is sodium saccharin. It induces bladder cancer only in rats, particularly in males, but not in mice, hamsters, or monkeys (12, 82). The tumorigenic effect of sodium saccharin is likely due to formation of silicates in the urine of male rats. Factors, including pH, protein, sodium, silicate, and saccharin, reach critical levels in the urine following feeding of high doses of sodium saccharin to rats. A threshold effect is likely. If lower doses of sodium saccharin are administered, silicate precipitates and crystals do not form, cell proliferation is not increased, and there is no increased tumor formation. Moreover, the critical set of urinary parameters in the rat following high doses of sodium saccharin is not present in humans, mice, or monkeys. Hence, humans appear to be resistant and would not be expected to develop bladder cancer even at extremely high doses of sodium saccharin.

Assessment of risk of chemical compounds is controversial. Regulatory agencies are determining whether differences should be made in interpreting data between nongenotoxic and genotoxic compounds. Genotoxic compounds generally do not appear to have a threshold regarding their genotoxic effects, but the tumorigenic response is greatly augmented at doses producing increased cell proliferation. In contrast, most (if not all) nongenotoxic compounds likely require a threshold dose for increasing cell proliferation, and consequently, they are likely to

have a no-effect threshold with respect to tumorigenesis. This is particularly true for nongenotoxic chemicals that act through a mechanism not directly involving a cell receptor.

Substantial difficulties arise in interpreting the carcinogenic potential of many chemicals, exemplified by asbestos (83). For example, controversy continues as to whether asbestos itself is genotoxic, since it has clastogenic activity in some cell culture systems. Also of significance, exposure of limited duration to asbestos is actually lifetime exposure, since it remains within the mesothelial cells. Thus, it provides a chronic proliferative stimulus even if the actual environmental exposure was relatively brief. The challenge remains to ascertain whether there is a minimal level of chronic proliferation that determines whether there is a no-effect threshold exposure level with respect to asbestos-induced carcinogenesis in humans.

CONCLUSIONS

We have presented a two-event model of carcinogenesis, wherein agents increase the likelihood of developing cancer by increasing the probability of genetic damage during each cell mitosis or by increasing the number of cell divisions subject to spontaneous genetic damage probabilities (*i.e.*, cell proliferation), or by doing both. Whether cancer occurs by two, or more, critical genetic events, these two general mechanisms remain as the only ones by which an agent can increase cancer risk. Agents causing direct genetic damage during cell division, such as radiation and genotoxic chemicals, are not likely to have a threshold for carcinogenic response. Further, the dose-response can be significantly influenced by the cytoxicity and regenerative hyperplasia that follow exposure to these agents at high doses.

For agents that act only by increasing cell proliferation, whether nongenotoxic chemicals, infectious organisms, or chronic inflammatory processes, the magnitude and duration of the increased proliferative processes are integral to carcinogenesis. Brief responses will probably not be associated with a detectable increased risk of cancer, since any increased proliferation will be of short duration and contribute little to the total number of cell divisions during which spontaneous genetic damage might occur.

Both genetic damage and increased cell proliferation act in human and in animal carcinogenesis. Study of the complex of biochemical and physiologic adaptive and maladaptive tissue processes offers numerous opportunities for enhancing our understanding of the carcinogenic process and for designing preventive intervention strategies.

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